



## Danger on the track? Tick densities near recreation infrastructures in forests

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### ABSTRACT

The risk of tick-borne disease in humans depends on the exposure to pathogen-infected ticks, which in turn is driven by local tick population densities, pathogen prevalence and human activity. Variation in tick densities and pathogen prevalence between green spaces differing in habitat characteristics, location and geography has been well documented. In contrast, variation within green spaces, although vital for management and prevention of disease risk, remains poorly understood. Studying this variation may lead to a better understanding of the drivers of small-scale tick distribution and reveal priority locations for tick management. We sampled ticks within green spaces at three location types representing different green space infrastructures and levels of human activity: (1) a structural element (e.g. bench); (2) the ecotone 40 m further along the adjacent trail and; (3) the interior of the associated forest stand, 20 m perpendicular to the trail, between (1) and (2). Drag sampling took place in 2018 and 2019 at 36 locations in 10 green spaces located in the Campine region of Flanders, Belgium. The density of questing nymphs (DON) was lowest at structural elements and slightly higher adjacent to trails. The highest tick densities were recorded in the forest interior. DON was higher in deciduous than in coniferous forests as well as in stands with a more developed shrub layer. This was true for all location types, as we observed a strong correlation of DON between location types within forest stands. This enables the prediction of DON within forest stands, thus enabling the prediction of DON near infrastructure based on the associated forest stand characteristics. Prevention and management efforts should be focused on infrastructure in or adjacent to deciduous, structure-rich forest stands, although large variation in DON at all location types indicates overall, factual risk while using green space infrastructure.

### 1. Introduction

In an urbanizing world with a growing human population, recreation pressure in green spaces will increase (Zipperer and Pickett, 2012) which can lead to a higher contact rate between people and wildlife, including vectors of zoonotic diseases (Medlock and Leach, 2015; Braks et al., 2016). In contrast to tropical regions, where mosquitos are the main vectors of zoonotic disease, ticks are the most common vectors in temperate zones (Mead, 2015; Stanek et al., 2012). Over the last decades, there has been an increase in the prevalence of tick-borne diseases (TBD) in Western Europe (Hofhuis et al., 2015; Smith et al., 2000). The most prevalent example is Lyme borreliosis, which is caused by the spirochete *Borrelia burgdorferi* s.l. The disease is widely viewed as the vector-borne disease with the highest healthcare and economic cost in Western Europe (Jongejan and Uilenberg, 2004; Stanek et al., 2012;

Sykes and Makiello, 2016).

The most notable vectors of TBDs, including Lyme borreliosis, in temperate zones are the generalist tick species of the *Ixodes ricinus* species complex (Keirans et al., 1999). In Europe, the most abundant species of tick is *Ixodes ricinus* (Linnaeus 1758, henceforth “tick”), which belongs to this complex and is commonly known as the castor bean tick or sheep tick (Piesman and Gern, 2017). The species complex includes also a.o. *I. persulcatus* and *I. scapularis*. These ticks are ectoparasites that have three active life stages: larva, nymph and adult. In order to progress to the following stage, they attach to a vertebrate host and take a single bloodmeal, detach and migrate down the litter layer where they molt into the subsequent stage. Usually, the life cycle lasts two to three years, during which most of the time is spent in the free-living phase (Gray, 1991; Randolph, 2004). Ticks from this complex exhibit questing behaviour in order to find a suitable host. The host-seeking ticks climb

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up a blade of grass or other structure and wait for a passing animal (Mejlon and Jaenson, 1997). Larvae predominantly feed on rodents and songbirds, while nymphs infest a wide variety of vertebrate hosts. Adult female ticks typically feed on a large mammal (Gray, 1998), and simultaneously get fertilized by available males. After detachment from the host, the female deposits on average 2000–3000 eggs and dies (Randolph, 2004, 1998; Buczek et al., 2019). Because of the high reproductive output, the availability of suitable hosts for adult females strongly determines the tick presence in forests and other green spaces (Gilbert et al., 2012; Hofmeester et al., 2017).

Infection of humans with TBDs occurs when a pathogen-infected tick feeds on a human. In case of the causative agent of Lyme disease, *Borrelia burgdorferi* s.l., there is no evidence of transovarial transmission. Thus, larvae usually acquire the pathogen through feeding on a bacteremic host or co-feeding with other, infected ticks and then bacteria are transmitted transstadially (Gern and Rais, 1996; Humair et al., 1999). Since adult ticks are less prevalent than nymphs and are easily noticed when infestation occurs, nymphs are most responsible for transmitting pathogens to humans and therefore the focus of this study. The local risk of infection with a tick-borne pathogen is commonly defined as the contact rate between infected ticks and humans, with the density of questing nymphs (DON) acknowledged as its main driver (Braks et al., 2016; Heylen et al., 2019; Ostfeld et al., 2006; Perret et al., 2004; Ruys et al., 2016).

Studies on drivers of DON have mainly focused on temporal variation and large-scale spatial variation. Seasonal patterns of questing activity are characterised by a peak in spring and early summer (“spring cohort”) and a second, smaller peak in late summer and autumn (“autumn cohort”) (Cayol et al., 2017; Gray, 1991; Randolph et al., 2002). The consistency of these patterns within the *Ixodes ricinus* species complex implies that temporal variation should be accounted for in the design of studies examining spatial variation in DON. On the other hand, studies on spatial variation have identified vegetation and landscape characteristics as drivers of variation in DON between green spaces (Ehrmann et al., 2017; Estrada-Peña, 2001; Medlock et al., 2013; Tack et al., 2012a, 2012b; Vourc’h et al., 2016). These spatial effects are explained by vegetation-related microclimate, which may affect tick survival during the nonparasitic phase, and the composition of the available host community (Daniel, 1993; Gehlhausen et al., 2000; Lees, 1946).

Contrary to these large-scale spatial and temporal effects, the variation in DON within forest stands is poorly understood. This information is, however, of vital importance for targeted management concerning tick-borne disease risk within green spaces. Moreover, visitors are not spread homogeneously in green spaces but spend most, if not all of their time near recreational infrastructure such as benches and trails. Few studies have been conducted on within-green space variation in DON (Dobson et al., 2011; Kiewra et al., 2017; Vourc’h et al., 2016) and the association of human infrastructure and management with tick abundances (Hansford et al., 2017; Medlock et al., 2013; Millins et al., 2017). So far, these studies have not explored fine-scale variation within forest stands associated with recreational infrastructure, nor did they evaluate the consistency of such patterns among green spaces and over time.

In this study, we aim to investigate the relation between green space infrastructure and local DON at within-forest stand scale, as we hypothesize that DON is lower near infrastructure compared to nearby forest locations. We therefore investigate (1) to what extent DON is affected by two different types of green space infrastructure (benches and trails) and; (2) whether these relations are consistent between forest stands and green spaces, and over time. This would allow for the prediction of local DON (and therefore tick-bite risk) near frequently used infrastructure, relative to other forest stands within the same green space. Such predictions would help identify priority locations for targeted management and sensibilisation.

## 2. Materials and methods

### 2.1. Study area

Tick collection was conducted in 10 green spaces located in the Campine region of Flanders, Belgium (Table 5 – Supplementary Materials). Sampling was conducted both in spring and autumn of 2018 and 2019, corresponding to the two peaks in tick activity (Cayol et al., 2017; Gray, 1991; Randolph et al., 2002). An additional sampling of each triplet was carried out in September – October of 2018 (Fig. 3- Supplementary Materials). All selected green spaces are publicly accessible for recreational purposes. The Campine region has a temperate climate with warm summers. During the sampling period, local annual mean temperatures ranged from 11 °C to 12.5 °C and total annual precipitation from 600 mm to 900 mm, evenly distributed throughout the year (Royal Meteorological Institute of Belgium, 2020).

### 2.2. Collecting of ticks: drag sampling design

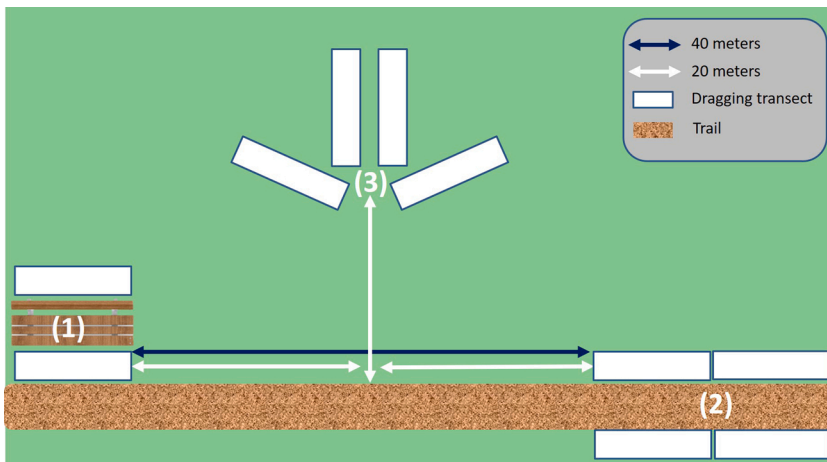
Questing *Ixodes ricinus* were collected by drag sampling litter and herb layer vegetation with a white flannel cloth (1 m<sup>2</sup>) for a distance of 5 m (MacLeod, 1932). This was done at 36 triplets, divided over the aforementioned green spaces (Fig. 4 and Table 6 – Supplementary Materials) each containing three location types: (1) an infrastructural element (mostly benches, also picknick tables or information boards, henceforth “bench”); (2) an adjacent unpaved trail between 1 and 3 m wide and; (3) a forest interior location (Fig. 1). Triplets were selected based on the availability of these three location types within 40 m from one another as described in Fig. 1. Dragging across bramble or large woody debris was avoided and sampling was not conducted on rainy days or when vegetation was wet. Collected ticks were identified based on morphological criteria and captured *I. ricinus* were stored in 70 % ethanol for later analyses of pathogen communities.

### 2.3. Environmental measurements

Air temperature and relative humidity were measured for each location type at the moment of sampling, since these variables are known to influence the questing behaviour of ticks (Heylen et al., 2013; Randolph, 2004). This was done at 5 cm above ground level and at 120 cm above soil level using a Kestrel 3000 pocket weather meter (Kestrel Weather & Environmental Meters, USA). Relative humidity was transformed to absolute humidity, as to avoid autocorrelation with temperature (henceforth “T5”, “AH5”, “T120” and “AH120”, respectively). For each triplet, the forest stand type (deciduous or coniferous) was determined based on whether the canopy was dominated by deciduous or coniferous vegetation. Also, the percentage herb cover and shrub cover were estimated in the forest interior. Based on the latter, each triplet was designated as structure rich (cover > 50 %) or structure poor (cover < 50 %).

### 2.4. Statistical analyses

Statistical analyses were performed in R version 3.5.2 (2018-12-20, The R Foundation for Statistical Computing, Austria, [www.r-project.org](http://www.r-project.org)). Generalised linear mixed models (GLMM) with a negative binomial error distribution were drafted with the `glmer.nb` function of the `lme4`-library (Bates et al. 2011) which was built in R 2.13.0. The density of questing nymphs (DON) per transect was defined as the response variable. Initial models included only space and time variables, i.e. the month and year of sampling, as well as the location type and interactions between these fixed variables. Green space and triplet were added as random nested variables. In the next step we included more fixed factors to increase the amount of variation in DON explained. The following fixed effects were added at triplet level, as well as their interactions with location type: forest stand type, structure richness and percentage herb



**Fig. 1.** Schematic overview of the dragging transects, taken at a triplet during each sampling event. For location type (1), at least one transect of 5 m<sup>2</sup> was sampled between the bench and the trail and one on the far side of the bench. Two additional transects were sampled whenever the size of the infrastructural element allowed for this, one on either side. At location type (2), four transects were sampled: two at either side of the trail. Drags were conducted on vegetation and litter directly adjacent to the trail, as this environment represents the highest chance of contact between visitors and ticks. At location type (3), transects were selected to be representative for the vegetation in the forest interior. These transects were chosen ad hoc as close as possible to a point designated 20 m perpendicular to the trail, midway between location types (1) and (2).

cover. Additionally, the same was done for microclimate variables at the location type level. The latter were standardized within months to avoid convergence issues with the factor month. Strong correlations were observed between T5 and T120, as well as between AH5 and AH120 (all Spearman correlation coefficients > 0.80). After comparing models with individual microclimate variables, T5 was included in the final model selection based on ΔAICC and individual p-value. Linear effect models were used to evaluate whether temperature or humidity varied significantly between location types. This was not the case. Model selection was performed by elimination of variables based on ΔAICC (Bolker, 2008).

We also explored to what extent DON adjacent to trails and benches could be predicted from DON in the forest interior. Mean DON was calculated for each sampling day per triplet and per green space, after which pairwise Spearman correlation coefficients were calculated between DON levels in the three location types across triplets. This was done for the full dataset, as well as datasets of the spring and autumn cohort, since the consistent predictability of DON near infrastructure is of interest to green space managers. To this end, we also evaluated the temporal consistency of the correlations through linear models with DON at bench and trail locations as a response variable. DON at the forest interior, the month of sampling and their interaction were added as explanatory variables, with green space and triplet as nested random effects.

**3. Results**

During the sampling period 1377 drag transects of 5 m<sup>2</sup> were sampled (6885 m<sup>2</sup>). In total, 4244 *Ixodes ricinus* nymphs (henceforth “nymphs”) were collected: 3095 in 2018 and 1149 in 2019, translating to densities of questing nymphs (DON) of 0.61 and 0.64 ticks per m<sup>2</sup>, respectively. Both the maximum and mean recorded DON were lower at bench locations compared to trails, where DON, in turn, was lower than in the forest interior (Table 1). At the bench location, nymphs were captured at only 49.84 % of transects, whereas this was the case for 59.34 % and 76.17 % of drags at trails and in the forest interior, respectively.

**Table 1**  
Observed densities of *Ixodes ricinus* nymphs per 1 m<sup>2</sup> at each location type.

|                  | Mean ± SD (Nymphs / m <sup>2</sup> ) | Min (Nymphs / m <sup>2</sup> ) | Max (Nymphs / m <sup>2</sup> ) |
|------------------|--------------------------------------|--------------------------------|--------------------------------|
| Across locations | 0.62 ± 1.35                          |                                |                                |
| Bench            | 0.30 ± 0.59                          | 0                              | 5.2                            |
| Trail            | 0.46 ± 0.77                          | 0                              | 7.4                            |
| Forest interior  | 0.98 ± 1.97                          | 0                              | 30                             |

Our initial GLMM, containing only time and space variables, demonstrated that the aforementioned differences in DON between location types were highly significant (Table 2). There were also significant differences in observed DON between months: higher densities were recorded in spring and early summer (May – July) compared to late summer and autumn (August – October) (Fig. 2). However, there was no significant interaction between location type and the month of sampling.

In the next model, including additional fixed effects, the effects of space and time variables described above remained significant (Table 3). We found a non-significant effect of forest stand type. Also, a near-significant (p = 0.06) effect towards higher DON in triplets located in structure rich forest stands was observed. None of the tested interactions between vegetation characteristics and location types were significant (all p > 0.44). The same significant effects as described in the initial model above persist, in addition to a significant effect of temperature at 5 cm above ground level with higher DON being recorded at lower temperatures (standardized within month).

The high variance associated with the triplet random effect implies

**Table 2**  
Description of parameter estimates resulting from the generalised linear mixed model investigating the effects of spatial and time variables on local density of questing nymphs per 1 m<sup>2</sup>. The marginal R<sup>2</sup> of the final model is 0.29 and the conditional R<sup>2</sup> 0.59.

| Fixed effects                 | F-statistic                    | Estimate ± SE            | p-value |
|-------------------------------|--------------------------------|--------------------------|---------|
| <b>Spatial variable</b>       |                                |                          |         |
| Location type                 | F <sub>1,18,25</sub> = 14.33   |                          | < 0.001 |
| Bench                         |                                | (intercept) -0.26 ± 0.22 |         |
| Trail                         |                                | 0.42 ± 0.14              | 0.0029  |
| Forest interior               |                                | 1.09 ± 0.20              | < 0.001 |
| <b>Time variable</b>          |                                |                          |         |
| Year and month                | F <sub>5,1175,77</sub> = 62.90 |                          | < 0.001 |
| 2018 June                     |                                | 0.98 ± 0.11              | <0.001  |
| 2018 July                     |                                | 0.70 ± 0.11              | <0.001  |
| 2018 August                   |                                | (intercept) -0.26 ± 0.22 |         |
| 2018 September                |                                | -0.85 ± 0.12             | <0.001  |
| 2018 October                  |                                | -0.66 ± 0.20             | 0.0011  |
| 2019 May                      |                                | 1.45 ± 0.13              | <0.001  |
| 2019 July                     |                                | 0.50 ± 0.16              | 0.0015  |
| 2019 September                |                                | -0.50 ± 0.15             | <0.001  |
| 2019 October                  |                                | -0.38 ± 0.23             | 0.10    |
| <b>Interaction</b>            |                                |                          |         |
| Month * Location type         | F <sub>6,912,50</sub> = 1.71   |                          | 0.052   |
| <b>Random effects</b>         |                                |                          |         |
|                               |                                | Estimate                 | p-value |
| Triplet nested in Green space |                                | 0.38 ± 0.61              | <0.001  |
| Green space                   |                                | 0.12 ± 0.35              | 0.63    |

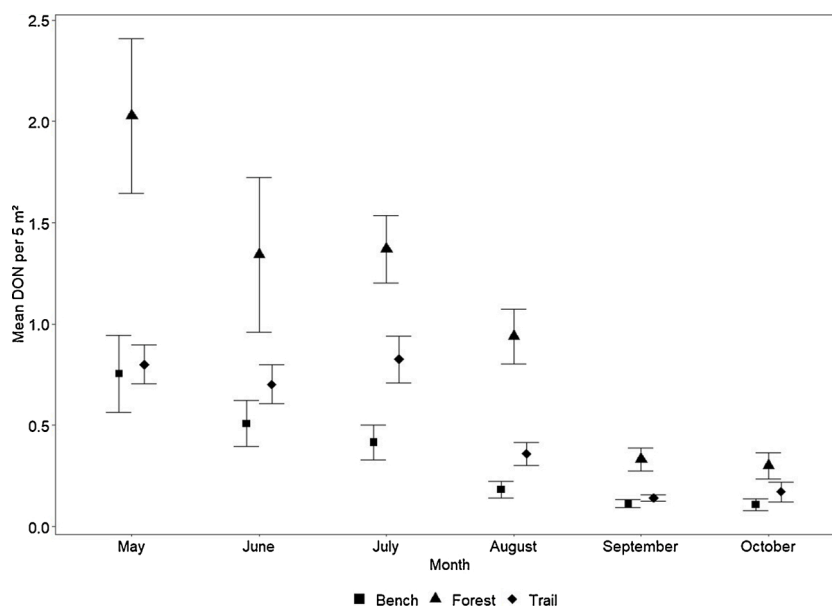


Fig. 2. Mean ( $\pm$  SE) of observed density of questing nymphs per 1 m<sup>2</sup> at each location type for all sampled months.

Table 3

Description of variables included in the final generalised linear mixed models used in this study to explain density of questing nymphs. Climate variables were standardised within year and month of sampling. Values of non-significant fixed effects are those before exclusion. The conditional R<sup>2</sup> of the final model is 0.57, the marginal R<sup>2</sup> 0.33.

| Fixed effects                  | F-statistic                  | Estimate $\pm$ SE            | p-value |
|--------------------------------|------------------------------|------------------------------|---------|
| <b>Spatial variable</b>        |                              |                              |         |
| Location type                  | F <sub>2,11374</sub> = 14.68 |                              | < 0.001 |
| Bench                          |                              | (intercept) -0.34 $\pm$ 0.24 |         |
| Trail                          |                              | 0.44 $\pm$ 0.14              | 0.0020  |
| Forest interior                |                              | 1.12 $\pm$ 0.20              | < 0.001 |
| <b>Time variable</b>           |                              |                              |         |
| Year and month                 | F <sub>8,1368</sub> = 98.83  |                              | < 0.001 |
| 2018 June                      |                              | 0.97 $\pm$ 0.10              | < 0.001 |
| 2018 July                      |                              | 0.72 $\pm$ 0.11              | < 0.001 |
| 2018 August                    |                              | (intercept) -0.34 $\pm$ 0.24 | < 0.001 |
| 2018 September                 |                              | -0.87 $\pm$ 0.12             |         |
| 2018 October                   |                              | -0.63 $\pm$ 0.20             | 0.0022  |
| 2019 May                       |                              | 1.46 $\pm$ 0.13              | < 0.001 |
| 2019 July                      |                              | 0.40 $\pm$ 0.15              | 0.009   |
| 2019 September                 |                              | -0.55 $\pm$ 0.15             | < 0.001 |
| 2019 October                   |                              | -0.36 $\pm$ 0.23             | 0.12    |
| Year and month * Location type | F <sub>10,1359</sub> = 1.67  |                              | 0.26    |
| <b>Climate variables</b>       |                              |                              |         |
| <b>Standardised monthly</b>    |                              |                              |         |
| Temperature (ground level)     | F <sub>1, 1131</sub> = 18.34 | -0.16 $\pm$ 0.037            | < 0.001 |
| <b>Vegetation</b>              |                              |                              |         |
| <b>Dominant tree species</b>   |                              |                              |         |
| Deciduous                      | F <sub>1,1375</sub> = 1.15   | (intercept) -0.34 $\pm$ 0.24 | 0.15    |
| Coniferous                     |                              | -0.43 $\pm$ 0.30             | 0.17    |
| <b>Structure richness</b>      |                              |                              |         |
| Structure poor                 | F <sub>1,1375</sub> = 4.68   | (intercept) -0.34 $\pm$ 0.24 | 0.061   |
| Structure rich                 |                              | 0.56 $\pm$ 0.29              | 0.057   |
| <b>Random effects</b>          |                              |                              |         |
|                                |                              | Estimate                     | p-value |
| Triplet nested in greenspace   |                              | 0.38 $\pm$ 0.61              | < 0.001 |
| Greenspace                     |                              | 0.12 $\pm$ 0.35              | 0.63    |

that DON in one location type is correlated with DON at the other two types within a single triplet, independently of fixed effects. To explore this further, DON was averaged for each sampling day, both on a triplet level and a green space level. The strongest correlations were found between forest and trail ( $r$  from 0.47 to 0.79, all  $p < 0.01$ ), but bench-forest and bench-trail correlations were also generally high (all  $r > 0.31$ ) and in most cases statistically significant (Table 4). Correlations were generally higher for green space means than for triplet means, but inevitably significance levels were lower for green space means due to the smaller sample size. Correlations were substantially lower in autumn than in spring in some, but not all cases (Table 4).

The GLMs analysing the relationship between DON in the forest interior and DON at infrastructure revealed a significant correlation, both for the bench and trail infrastructure types (Table 7 – Supplementary Materials). The marginal R<sup>2</sup> for the models predicting DON at the bench and trail location types were 0.38 and 0.31, whereas conditional R<sup>2</sup> values were 0.44 and 0.51, respectively. Therefore, a large part of the explanatory power of the models is attributed to the fixed effects, such as DON in the forest interior as well as the sampling year and month. There was also a significant interaction between the two fixed variables for the model predicting DON at the bench.

Table 4

Overview of correlation in mean density of questing nymphs between location types with means taken at triplet level and at green space level per sampling day. For means taken at triplet level, samples size is 137, for means taken at green space level 54.

|   |        | Entire year |         | Spring cohort |         | Autumn cohort |         |
|---|--------|-------------|---------|---------------|---------|---------------|---------|
|   |        | Cor. coef.  | p-value | Cor. coef.    | p-value | Cor. coef.    | p-value |
| <b>Correlation of triplet means</b>     |        |             |         |               |         |               |         |
| Bench                                   | Forest | 0.48        | < 0.01  | 0.32          | 0.02    | 0.39          | < 0.01  |
| Bench                                   | Trail  | 0.59        | < 0.01  | 0.64          | < 0.01  | 0.31          | < 0.01  |
| Forest                                  | Trail  | 0.66        | < 0.01  | 0.47          | < 0.01  | 0.53          | < 0.01  |
| <b>Correlation of green space means</b> |        |             |         |               |         |               |         |
| Bench                                   | Forest | 0.60        | < 0.01  | 0.59          | < 0.01  | 0.37          | 0.09    |
| Bench                                   | Trail  | 0.72        | < 0.01  | 0.74          | < 0.01  | 0.41          | 0.06    |
| Forest                                  | Trail  | 0.79        | < 0.01  | 0.65          | < 0.01  | 0.67          | < 0.01  |

#### 4. Discussion

The densities of questing nymphs (DON) reported here, with an overall mean of 0.62 nymphs per m<sup>2</sup> and a mean of 0.98 nymphs per m<sup>2</sup> in the forest interior, are comparable to other West-European studies in which similar habitat was monitored (0.42–1.44 nymphs per m<sup>2</sup>) (Dobson et al., 2011; Heylen et al., 2019; Medlock et al., 2012; Vourc'h et al., 2016). Nevertheless, our estimates of local nymphal densities are higher than previous studies in Flanders that reported 0.20 to 0.45 nymphs per m<sup>2</sup> (converted from reported DON at 100 m<sup>2</sup>) (Ruyts, 2017; Tack et al., 2013, 2012b, 2012a). This may be a consequence of our dragging transects being shorter, since vegetation and litter may scrape off attached ticks.

Within this study, we have put forward two research questions concerning the fine-scale spatial DON. First, we confirmed that DON is lower near infrastructure based on the outcomes of our GLMMs. More specifically, we found that DON is approximately 50 % lower at trails compared to forest interior, and another 30 % lower at benches. Secondly, we confirmed that these location type effects were consistent over time and between environments. Given the significant correlation coefficients between DON at individual location types for each cohort, the density of nymphs at trails and benches can be predicted from the characteristics of the associated forest stand.

Due to the limited intrinsic mobility of *Ixodes ricinus*, it is likely that the large scale drivers of DON also act on the small spatial scales we investigated. Several explanations for the differences in DON between location types can be put forward, but all link up with the basic conditions for *I. ricinus* survival and propagation: suitable microclimate conditions and the presence of hosts on which larvae can feed. The importance of microclimate, which is shaped by soil characteristics and vegetation, lies in the fact that *I. ricinus* requires a relative humidity of 80 % for its survival and propagation (Daniel, 1993; Kahl and Knülle, 1988). Therefore, a humid and temperate microclimate increases tick survival (Medlock et al., 2008; Nelson et al., 2015; Randolph and Storey, 1999). This explains why higher DON persists in the forest interior, since infrastructure forms internal forest edges and the resulting edge effect dictates higher soil moisture and air humidity in the forest interior, compared to forest edges (Gehlhausen et al., 2000; Schmidt et al., 2017; Smith and Johnson, 2004). This is mainly due to the buffering effect of the canopy (De Frenne et al., 2019). Additionally, soil near infrastructure is often more compressed, making it less permeable for water and increasing runoff. The open spaces created by infrastructure increase exposure to wind and direct sunlight, facilitating evaporation and the desiccation of questing ticks, thus reducing survival and questing activity (Medlock et al., 2008).

Habitat use of the tick hosts may further explain the observed infrastructure effect. Suitable hosts for adult females, which in the Flanders region are predominantly large ungulates such as roe deer, spend most of their time in the forest interior: either foraging for food or at well covered, undisturbed resting sites (Mysterud, 1996; Tufto et al., 2020). Consequently, the lion share of fed adult female *I. ricinus* will detach and lay eggs in the forest interior. Nymphs end up near infrastructure when they detach from larval hosts that use the ecotone near benches and trails. It is likely that many larval hosts spend less time near infrastructure as they are deterred by humans and avoid the open vegetation types created by management (Miller and Hobbs, 2000; Miller et al., 1998; Pasinelli et al., 2016; Riber, 2006; Westekemper et al., 2018). More research is therefore needed on small-scale habitat use of larval hosts and its correlations with DON. In addition, local host community composition will affect the nymphal infection prevalence (NIP) through differences in reservoir competence for different pathogens of specific host species (LoGiudice et al., 2003; Rosà and Pugliese, 2007; Ruyts et al., 2016). In the case of *Borrelia burgdorferi* s.l., rodents have been identified as the main reservoir for several genospecies causing disease in humans, and songbirds have been designated as the reservoir for the *Borrelia garinii* genospecies, which is the causative agent

of neuroborreliosis (Heylen et al., 2014; Hofmeester et al., 2016; Krawczyk et al., 2020; LoGiudice et al., 2008; Matuschka et al., 1992). Although DON is generally considered as the main driver of the density of infected nymphs (DIN), and thereby the contact rate between humans and infected nymphs (Braks et al., 2016), more research into the correlations between NIP and green space infrastructure may further improve our understanding of the risk for green space visitors.

In addition to the abovementioned factors, depletion of local tick populations by attachment of ticks to passing humans and their pets could partly explain the observed differences between location types. However, this effect would only apply to very frequently used trails and other infrastructure types where visitors and their pets spend considerable amounts of time. In order to test whether such a depletion effect occurs, detailed information on tick density, contact rates and time spent by visitors is needed.

Besides the effect of location type, we found significant differences in DON among months and years. Similar intra-annual variation has been described in other European studies, with higher densities in spring cohorts compared to autumn cohorts (Cayol et al., 2017; Gray, 1991; Randolph et al., 2002). Differences between years can have biotic causes, such as fluctuations in host population densities (Ostfeld et al., 2006). A prominent and much debated example are the fluctuations in tick densities due to differences in food availability for host populations in masting years (Krawczyk et al., 2020; Randolph, 1998; Tack et al., 2013). Abiotic factors such as weather fluctuations may also have an effect on measured DON (Gray, 2008; Randolph, 2010). Similar factors drive variation in measured DON over shorter time intervals. This explains the observed temperature effect in our study, relative to the mean monthly temperature recorded. The negative estimate is explained by the questing behaviour of ticks: they climb the vegetation and are subject to desiccation. With higher temperature, the drying potential of the air increases, reducing the time ticks can spend questing (Knülle and Rudolph, 1982; Sonenshine, 1991).

Variation between triplets is partly explained by the vegetation characteristics of the forest stand. Here we see an effect of the development of the shrub layer, where more structure rich stands are associated with higher DON. The same is true for deciduous forest stands, compared to predominantly coniferous stands. These observations confirm that the correlations between DON and vegetation characteristics between green spaces, as described in the current literature, are also applicable within green spaces (Lindström and Jaenson, 2003; Ruyts et al., 2018; Tack et al., 2012c, 2012b). As mentioned above, vegetation has a direct effect on survival through by creating a humid microhabitat. In addition, it also determines habitat suitability for hosts, indirectly effecting DON through host densities.

Additional variation is explained by the random effects in our models. Here, high variation among triplets within the same greenspace was observed, while on average the overall DON between greenspaces did not differ that much (see random effect estimates Table 3). We may wonder whether this within-forest patchiness can be further explained by not yet defined habitat quality characteristics and time-related variables, or whether the patterns have a merely stochastic nature making it hard to predict local DON. A few adult ticks detaching in the same location may generate local clusters of thousands of larvae which may still persist in the nymph stage, given that larvae feed on hosts with small home ranges such as rodents (Mejlon and Jaenson, 1997). Further research on the drivers of small-scale variations in DON is needed.

The findings of our study can help to plan management actions to reduce the tick exposure risk. Given that humans spend most of their time in green spaces at infrastructure, management should be prioritized at infrastructure in structure rich forest stands with predominantly deciduous vegetation. Actions can either target a reduction of DON (e.g. sheep mopping (van Wieren, 2016)) or a reduction of the contact rate between questing nymphs and humans (Braks et al., 2016). The latter can be done by altering vegetation structure (e.g. mowing), since vertical structure of vegetation increases the contact probability with ticks

(Verheyen and Ruyts, 2016). Another way to reduce contact rate is through sensibilisation, which should focus on encouraging visitors to stay on trails, since DON is highest in the forest interior. However, the large variation in DON at all location types, with peak counts of 5 nymphs per m<sup>2</sup> even at benches, implies that visitors should also stay cautious while using green space infrastructure. The interpretation of the temporal variation in DON can further instruct management protocols. Management efforts in spring and early summer (the first cohort of tick activity) should be prioritised over management efforts in mid-summer and autumn, if necessary.

## 5. Conclusion

We demonstrated an effect of green space infrastructure on the fine-scale distribution of the sheep tick, *Ixodes ricinus*. Density of nymphs (DON) was lowest at frequently used infrastructural elements, slightly higher adjacent to trails and highest in undisturbed forest interior. We found high variation of DON between forest stands, driven by stand characteristics. Given the consistency of the infrastructure effect, the vegetation characteristics can be used to predict DON at trails and other infrastructure, relative to other locations within the same green space. This allows for the designation of priority locations with respect to the management of tick bite risk, based on the dominant tree species and structure richness of the associated forest stand. Infrastructure associated with deciduous, structure rich forest stands should thereby be prioritised. However, because nymphs were found at all types of locations we conclude that the risk of tick bites is omnipresent for recreants using green spaces, and emphasize the need for promoting public awareness.

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## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.ufug.2021.126994>.

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